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Efficacy of phototherapy to treat facial aging when using a red versus amber LED: a protocol for a randomized controlled trial

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ABSTRACT

Introduction: The skin undergoes morphological and physiological changes with the advancing age of the individual. These changes may be caused by intrinsic and extrinsic factors that contribute to cellular aging and consequent skin aging. The term photoaging is used to characterize the aging of the skin that is caused by solar radiation. Clinically, the skin becomes more flaccid, thicker, and hyperpigmented, while there is an early appearance of wrinkles and other skin changes, such as skin cancer. Nowadays, there are numerous treatments for aging skin and one of them is with the use of phototherapy which uses light emitting diodes (LEDs). LED devices can be found at various wavelengths, each of which can have different effects on the body. The objective of this study will be to evaluate the percentages of reduction of the volume of periocular wrinkles when treated with red and amber LEDs. **Methods and Analysis:** All of the participants will receive photobiomodulation in order to treat their periocular wrinkles. They will be using red and amber LEDs, with one color being used on each hemiface. The facial side to be treated with each color will be randomized to Group A, who will receive a red LED on the right side of their face and an amber LED on the left side of their face; Group B will receive a red LED on the left side of their face and an amber LED on the right side of their face. The complete treatment will consist of 10 sessions. After an interval of 180 days, the participants will receive a cross-treatment. The primary variable of the study is the volume of periocular wrinkles (crow's feet) that will be measured by VisioFace® equipment. The secondary variables are elasticity / flaccidity, which will be measured by Cutometer® equipment, as well as hydration, which will be measured by Corneometer® equipment. Their quality of life, as well as the self-assessment of the participants, will be measured by using the adapted MelasQol-BP and Skindex-29 questionnaires. All of the variables will be measured before and after the group of 10 sessions. **Ethics and Dissemination:** This protocol was approved by the Research Ethics Committee of the Nove de Julho University on June 21th, 2017 (# 2.134.166). **Registration:** This trial has been registered in the Registro Brasileiro de Ensaios Clínicos (Brazilian Clinical Trials Registry) (REBEC Number: RBR-6YFCBM, registered on July 19th, 2017). This study is not recruiting yet.

Keywords: Skin aging, wrinkles, phototherapy, photobiomodulation, photodermatology.

WORD COUNT: 3533

STRENGTHS AND LIMITATIONS OF THIS STUDY:

- Each woman participating in this study will be evaluated before and after the treatment and the reduction of wrinkles will be measured;
- There is no control or placebo group, all of the participants will be treated. In this sense, each participant is in both the treatment group and the control group;
- This split-face study will eliminate the individual factors of each participant on the treatment outcomes;
- Analyzes of the skin will be performed by non-invasive methods (no biopsy);
- The VisioFace® equipment will standardize the parameters of acquiring the photographs, such as light exposure, and it will minimize bias;
- The habits of the participants may affect the results, as a consequence of their diet, their cosmetics use and their exposure to the sun.

INTRODUCTION

The skin covers the body and it has essential functions in order to maintain the homeostasis of the organism, presenting roles of defense, thermoregulation and sensory awareness. The maintenance of healthy skin and integrity are both extremely important.(1) Exposure to the sun speeds up the intrinsic ageing of the skin, due to the formation of free radicals and reactive oxygen species, as a result of UV radiation (1). Once UVA radiation penetrates deeper into the dermis, the resulting oxidative stress causes damage to the elastin fibers and collagen. In addition, there may occur a decrement of physiological antioxidant reserves and/or of a protective capacity of the skin.(2) The changes that are caused by aging modify the physical properties of the skin, leaving visible signs, such as epidermal hyperplasia, irregular pigmentation, telangiectasia, sagging tissues, a reduction of collagen and the elastin fibers, as well as a decrement of the natural moisturizing factor (NMF). These changes cause the appearance of expression lines and creases.(3,4)

Recent data from the Brazilian Institute of Geography and Statistics (IBGE) has shown that the average life expectancy of the Brazilian population has increased from 66 years in 1991 to 75 years in 2016. This is similar to the increase that has been verified on the worldwide population. The challenge faced by science in the last few years has been the development of procedures and technologies, with the aim of delaying the signs of aging and increasing the quality of life of elderly people, by achieving healthy skin.(5) Nowadays, the procedures in use aim to promote not only a cosmetic benefit, but also an improvement in the quality of the skin, increasing self-esteem, with a reduction of skin infections. As a result, these procedures can contribute to a longer and healthier life. Among the technologies being used to promote skin repair are dermo-cosmetics, as well as equipment, such as radiofrequency, phototherapy (intense pulsed light, LASER and light emitting diodes - LEDs) and microneedles.(6–9)

Phototherapy is a non-invasive procedure that has been used for tissue repair and healing.(10,11) The treatment is based upon the use of a light emitting device and the resulting pho-

tons are absorbed by the biological tissues, promoting photochemical, photophysical and photobiological actions. Phototherapy is not ablative, nor does it promote thermal effects, since the devices that are used in phototherapy are low powered LASERs and LEDs; i.e., there is no cutaneous damage and no need for any recovery time.(12) The LED devices are produced in a wide range of wavelengths, from UV through the visible to infrared spectrum (247 to 1300 nm). When compared to LASER, the LED devices have a lower cost and the practicality of being used in instruments that can illuminate larger surfaces. Studies have shown that LEDs can be used in therapeutical procedures with excellent results.(10,13,14) The use of LEDs in clinical practice has increased significantly and their main use has been in wound healing, tissue repair and rejuvenation, since they do not cause trauma or tissue destruction.(15) Some findings have suggested that if suitable parameters are used, the light acts on skin regeneration, by modulating cellular activity and collagen expression, with a decrease of the matrix metalloproteinases (MMP).(16) Usually, the wavelengths are chosen by the function that is needed for the purpose of the therapy. The wavelengths in the blue range (400 to 470 nm) are mainly used in the treatment of acne.(17) The wavelengths in the green range (500-570nm) have shown their ability to induce a proliferation of fibroblasts, as well as in the production and maturation of the collagen fibers.(18,19) The infrared range (700 to 1200nm) accelerates the healing process of lesions in the skin, increases the proliferation of cell differentiation, as well as contributing to an increase in the extracellular matrix.(20,21)

Many *in vitro, in vivo*, and clinical studies have demonstrated the anti-inflammatory, repair, skin rejuvenation and healing effects that are promoted by red light.(11,22) For an amber light, a study that was published by Smith in 2005 showed that it has an affinity for keratinocytes, melanocytes, as well as for the cells of Merkel and Langerhans, which are all of extreme importance in the maintenance of the epidermis.(23) Both of these wavelengths are absorbed by cytochrome c oxidase, however, it is considered that the red light penetrates deeper into the skin than does the amber light, due to the presence of melanin.(24–26) In Brazil, the companies selling LED equipment for aesthetic

treatments claim that the amber light stimulates the ribosomes of fibroblasts, leading to an increased collagen synthesis, a stimulation of cell metabolism, in addition to skin hydration. The main amber LED equipment that is marketed for aesthetic professionals is distributed by Elite (DMC, Florida - USA), Fluence (HTM Eletrônica, Amparo - Brazil) and Venus (MMO, São Carlos - Brazil). All of this equipment is sold with an appealing charm for an improvement of the cosmetic conditions of the skin. However, there are no studies that have proven these cosmetic biological effects, especially when related to amber light. Given this background, this work will aim to evaluate the percentages of reduction of the volume of periocular wrinkles when treated with red and amber LEDs.

METHODS AND ANALYSIS:

Study Design

This will be a controlled, randomized, double-blind, split-face, crossover, and unicentric clinical trial. This protocol has been written based upon the SPIRIT guidelines. The study will be performed in the ambulatory of Biophotonics at the Nove de Julho University (UNINOVE), São Paulo, Brazil. Dissemination and registration for participation in the study will be conducted through the website of the University of Nove de Julho (UNINOVE) and the recruited participants will mainly be residents of the city of São Paulo. The participants will be informed about the research, the procedures, the risks and the benefits and they will sign the informed consent form**.** The study was approved by the Research Ethics Committee of the Nove de Julho University on June 21th, 2017 (# 2.134.166). Only those participants who have read and have agreed to sign the informed consent form will be included in the study. The study will last for 2 years, with a start date of April 2018. The study is not recruiting yet.

Sample Size Calculation

A pilot study with 10 patients was performed in order to generate the data for the sample size calculation. All of the participants of this pilot study signed the informed consent form. The largest

and the smallest values of the percentages of reduction in the volume of the wrinkles for each treatment were obtained, as well as for the standard deviation of the measurements. The worst case scenarios were used for this calculation. The smallest and the largest values were 95 and 5, respectively; the highest standard deviation was 29 and the number of treatment groups was 2. These values were used for the calculation of the effect size, as follows:

$$\Delta = \frac{largest - smallest}{\left(\frac{\sigma}{\sqrt{n}}\right)^2} = \frac{95 - 5}{\left(\frac{29}{\sqrt{2}}\right)^2} = 0.214$$

By using the effect size value as calculated above, t-tests were used to evaluate the differences between the two dependent means (matched pairs); the test power was 80% and the one-tailed test was at a figure of 5%; the sample size calculated by G*Power Software (Version 3.1.9.2, Dusseldorf, Germany) was 137.

Inclusion Criteria

This study will be conducted on women (40 to 65 years old), with skin phototypes II, III and IV on the Fitzpatrick scale and with signs of aging III and IV, on the Glogau scale.

Exclusion Criteria

This study will exclude any participants:

- With thyroid disorders (hyperthyroidism or hypothyroidism) and who are not undertaking the due treatment; or those that have been taking the medication for less than 1 year;
- Who have received a facial filling in the last 12 months;
- Who are doing any facial aesthetic procedure;
- Who are using retinoic acid or any vitamin A derivative (tretinoin, or isotretinoin, topical, or oral);

- Who are using cosmetics or medications that may increase the photosensitivity of the skin;
- Who present any pathology of the skin, such as acne, psoriasis, vitiligo, and so forth;
- Who have undergone bariatric surgery or who are confined to a strict diet;
- Who are using any supplement (topic or oral) for the improvement of their skin condition;
- Who are pregnant or lactating;
- Who are not regular attendees for the treatments.

Randomization

The equalized randomization will be performed by a researcher (ACRTH) who is not directly involved in the treatment of the participants. It will be generated in Excel 2013 software (Microsoft, USA). The opaque envelopes will be marked and identified by sequential numbers and each envelope will receive a paper containing the information of which particular treatment will be performed in the right hemiface of the participant, in accordance with the draw. These envelopes will be sealed and securely stored in a safe place, under the utmost confidentiality, by the same researcher who generated the randomization. Immediately before the treatments, the researcher responsible for the treatment will receive the envelope, in sequence, and will then perform the indicated procedure.

Interventions

All of the participants will have their faces cleaned with a neutral cleansing soap and receive eye protection, followed by the LED application. The participants will have their eyes protected by goggles, in order to safely allow for the illumination of their periocular region. This will also make the study blind, so that they do not know which wavelength is being applied to each hemiface. The application of phototherapy and the measurement of the parameters will be performed by ICSSR. Thus, this protocol will be a double-blind study.

All of the participants will receive photobiomodulation, in order to treat their periocular wrinkles, by using red and amber LEDs, with one color only at each hemiface. The facial side to be treated with each different color will be randomized. Group A will receive a red LED on the right side of their face and an amber LED on the left side of their face; Group B will receive a red LED on the left side of their face and an amber LED on the right side of their face. For both of the groups, the session will last for 10 minutes (5.4 J / cm² at each wavelength) and the complete treatment will be composed of 10 sessions, 2-3 sessions per week, within 1 month. After a period of 180 days, the crossover treatment will be performed: the participants in Group A will receive the application of an amber LED on their right hemiface and a red LED on their left hemiface, while the Group B participants will receive a treatment with a red LED on their right hemiface and an amber LED on their left hemiface. As performed in the first part of the study, both of the groups will have 10 minutes of exposure per session (5.4 J / cm² at each wavelength), with a complete treatment of 10 sessions, performed 2-3 sessions per week, within 1 month. All of the variables will be measured before and after the group of 10 sessions. This procedure will be conducted for each hemiface.

The participants may not receive any other facial aesthetic procedure or any supplement (topic or oral) for the improvement of their skin condition during the development of this study.

Variables of the Study

The primary outcome of the study is the volume of wrinkles in the periocular region. The secondary outcomes are elasticity / sagging, hydration, melanin/spots, the quality of life, and the self-assessment by the participants.

The volume of wrinkles in the periocular region and the melanin spots: For the measurements of the primary variable, VisioFace® - RD (CK Electronic, Cologne, Germany) equipment will be used. This apparatus has a digital camera with white diode illumination that will record a standardized

photograph of each participant's face. Through a computer program, parameters will be determined that will indicate the volume of wrinkles in the periocular region, which is commonly known as crow's feet.

Elasticity / Sagging: Other noninvasive measurements of the facial region will also be performed. The viscoelasticity of the skin will be evaluated in the periocular region by Cutometer® dual MPA 580 (CK Electronic) instrumentation. Cutaneous elasticity is an important parameter, because it provides information regarding the quality and quantity of collagen and elastic fibers (structural fibers) that are degraded by the metalloproteinases. It is known that photoaged skin presents disorganized elastin and decreased collagen fibers.(27)

Hydration: Skin hydration will be evaluated by a Corneometer® - CM 825 (CK Electronic) probe. This parameter is related to the amount of water in the dermis and epidermis, which allows for suitable skin functions.(28)

Quality of life and self-assessment by the participants: The participants will respond to the Quality of Life and Self-Assessment Questionnaires. An interview of around 20 minutes will be enough to get all of the participant's answers. In terms of the quality of life, an adaptation of the questionnaire for the quality of life of participants with dermatological diseases will be used.(29) This adapted questionnaire will also be used as the quality of life questionnaire for those participants with melasma - MelasQol-BP.(30) Due to the adaptations, these questionnaires will be evaluated in terms of reproducibility and internal consistency. For this, 20 participants, external to the main research, will respond to the questionnaires twice, with an interval of 30 days between the answers. Statistical analyzes will then be conducted. These particular participants will be duly informed about the research and, if they agree to participate in the study, they will sign the terms of free and informed consent.

The questionnaire results for any rhytidectomy will be performed before and after the phototherapy treatments for the verification of the self-assessment by participants.(30)

All of the measurements will be performed by LRM, who was previously trained by the CK Electronic's representative in Brazil. The VisioFace® equipment has standardized illumination and face positioning in order to minimize any experimental bias. The questionnaires will be applied by LRM.

The data that will be collected at this study will be managed only by the principal investigators (authors of this paper). The data will be saved on the University computer, protected by password.

Statistical Analyzes and Data Analyzes Plan

The Shapiro-Wilk test will be used to test for the normality of the data. If the data is non-parametric, the normalization will be performed by Math Strategy. The Student's t-test for dependent variables will be used for the inferential analyzes. A p-value < 0.05 will be considered statistically significant. DFTS will perform all of the statistical analyzes.

<Figure 1>

Discussion

Despite the fact that phototherapy has been proposed as an interesting tool to reduce wrinkles, clinical trials evaluating any real effects are sparse.(11,31,32) Regarding this subject, this study's protocol was designed in order to evaluate the reduction of wrinkles when using red or amber LED devices. This current work has described a study protocol for a unicentric randomized clinical trial based upon the comparison of two interventions. The study has been designed in order to optimize the obtainment of results and to minimize bias. Firstly, the participants will be their own control, since the measurements are going to be accomplished before and after a series of 10 sessions of in-

terventions. Secondly, by performing a split-face study, this will eliminate the individual factors of each participant on the treatment outcomes. By being aware of preexisting systemic pathologies, personal daily care and food intake, together with smoking and drinking habits, may affect the results obtained. As a result, by performing both treatments on each patient, this will make these factors influence the results equally for the two groups. In addition, if the application of phototherapy generates some systemic effects, it will have the same influence on the results of the two treatments. This has been carefully considered, since some studies have shown systemic effects of phototherapy.(33,34).Thirdly, a double-blind study will reduce errors of bias due to the subconscious influence of the volunteers, as well as the researchers on data acquisition. Finally, this randomization will be performed in an equalized way *(i.e., Group A = Group B)*, then, in the case of a patient's withdrawal after the first randomization, new inscriptions and a new randomization can be generated, thus allowing for the researchers to reach the desired number of patients for the study.

The aesthetics of the face may have positive or negative effects on the quality of life of the patients, as well as on their self-esteem. Despite being treated as futilities, aesthetic treatments may have strong and important influences on psychological and emotional levels, as well as on the well-being of the people. Some studies have already shown improvements in the quality of life and the self-assessment by patients after aesthetic treatments.(35,36) In this sense, the aforesaid questionnaires will be used in order to evaluate these aesthetic effects. Since the quality of life and the self-assessment questionnaires that have been validated in Portuguese when related to wrinkles are sparse, we decided to make fairly minor adaptions on some previously validated ones. The adaptation of the quality of life questionnaire for participants with dermatological diseases (29) has involved the removal of 10 questions related to pathologies of the skin that are not suitable for participants with wrinkles, resulting in a new questionnaire with 19 questions. The adaptation of the quality of life questionnaire for wrinkles and for those participants with melasma - MelasQol-BP (30) has included the substitution of the word 'melasma' instead of the word wrinkles. Due to these adapta-

tions, these questionnaires will be evaluated in terms of reproducibility and internal consistency, as has been performed on other questionnaires previously.(37)

When considering the importance of aesthetics of the face on the life role of a person, the development of efficacious treatments are essential. However, in order to prove that a treatment presents efficacy, the choice of a quantitative evaluation method is challenging. Most of the trials evaluating facial skin that can be found in the literature are based upon subjective measurements (patient satisfaction and photos), since quantitative studies use biopsies.(31,32,38) Here, for this research, non-invasive quantification methods will be used aimed at evaluating a group of variables that may be affected or improved by photobiomodulation therapy (volume of wrinkles, elasticity/sagging and hydration).

Some studies have pointed to the efficacy of LEDS in tissue repair, cutaneous hydration and an increase in the production of the sustentation fibers. However, there are still studies that standardize dosimetry and the parameters of use.(39,40) Here, the choice of wavelengths was made from studies that had demonstrated that an application of red light on the skin could trigger cell proliferation, increased collagen fibers and decreased metalloproteinases; these studies had also demonstrated that an amber light can interact with the epidermal cells, triggering mitosis and cell renewal, as well as acting on the protection and the hydration of the epidermis; all of this information was together with clinical trials showing an improvement of wrinkles by using phototherapy.(11,15,22,32,41) There is no consensus in the literature regarding the washout period for phototherapy, which varies from 7 days to 12 weeks.(42–45) Due to this, a washout of 180 days for performing the crossover treatment was chosen, in order to evaluate if there were any residual effects from the first photobiomodulation treatment performed.

The results of this clinical trial may confirm the efficaciousness of phototherapy in reducing periocular wrinkles and show improvements of certain other parameters. Besides, the comparison

between the reduction of wrinkles achieved by each wavelength may be a valuable contribution to the aesthetics area and on the way to developing new treatment protocols, with satisfactory results.

ETHICS AND DISSEMINATION:

This protocol was approved by the Research Ethics Committee of the Nove de Julho University on June 21th, 2017 (Acceptance Number: 2.134.166). The trial has already been registered in the Registro Brasileiro de Ensaios Clínicos (REBEC Number: RBR-6YFCBM, registered on July 19th, 2017) and it grants public access to the full protocol. After publishing the protocol, the data will be collected and the results will be presented at conferences and published in a peer-reviewed Journal, selected by interest area and impact factor. The authorship of the results paper will include the authors of the protocol and others that may contribute to the procedures or analysis of data.

AUTHORS' CONTRIBUTIONS:

LRM wrote the protocol and will execute the measurements. LJM adapted the QOL and the self-assessment questionnaires and will perfom the reproducibility and internal consistency analysis and LRM will apply them to the participants. DFTS performed the sample size calculation, proposed the statistics and will analyze the data. ACRTH generated the randomization and designed the study. CP conceived and designed the treatment protocol. All of the authors have read and approved the final version of the protocol and of the manuscript.

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COMPETING INTERESTS STATEMENT:

The authors declare the absence of any conflicts of interest.

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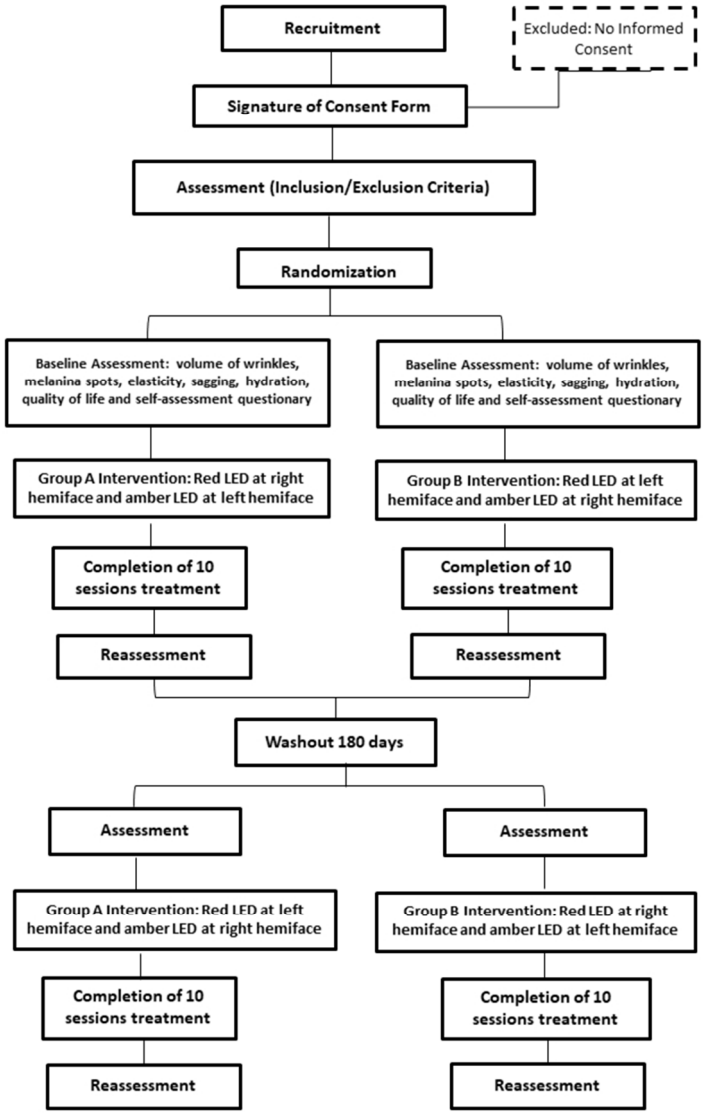
Figure Captions:

Figure 1: Study Timeline.



Study Timeline.

170x279mm (96 x 96 DPI)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	Page 1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	Page 2, 15
	2b	All items from the World Health Organization Trial Registration Data Set	N/A
Protocol version	3	Date and version identifier	Page 2
Funding	4	Sources and types of financial, material, and other support	N/A
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	Page 1 and 15
	5b	Name and contact information for the trial sponsor	N/A
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	N/A
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	Page 2
	6b	Explanation for choice of comparators	Page 5, 6
Objectives	7	Specific objectives or hypotheses	Page 2
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	Page 6
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	Page 6
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	Page 7, 8
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	Page 8, 9
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	N/A
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	Page 9, 10, 11
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	Page 9
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	Page 9, 10, 11
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Page 12

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	Page 7
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	Page 6
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	Page 8
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	Page 8
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	Page 8, 9
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	Page 8, 9
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	Page 9, 10, 11
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	N/A

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	Page 11
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	Page 11
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	Page 10, 11
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	N/A
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	N/A
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	N/A
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	Page 8, 9, 10, 11
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	Page 2, 15
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	N/A

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	Page 6
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	Page 6
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	Page 16
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	Page 15
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	Page 15
	31b	Authorship eligibility guidelines and any intended use of professional writers	Page 15
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	Page 15
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Page 6
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

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Efficacy of phototherapy to treat facial aging when using a red versus amber LED: a protocol for a randomized controlled trial

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Efficacy of phototherapy to treat facial aging when using a red versus amber LED: a protocol for a randomized controlled trial

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ABSTRACT

Introduction: The skin undergoes morphological and physiological changes with the advancing age of the individual. These changes may be caused by intrinsic and extrinsic factors that contribute to cellular aging and consequent skin aging. The term photoaging is used to characterize the aging of the skin that is caused by solar radiation. Clinically, the skin becomes more flaccid, thicker, and hyperpigmented, while there is an early appearance of wrinkles and other skin changes, such as skin cancer. Nowadays, there are numerous treatments for aging skin and one of them is with the use of phototherapy which uses light emitting diodes (LEDs). The objective of this study will be to evaluate the percentages of reduction of the volume of periocular wrinkles when treated with red and amber LEDs. **Methods and Analysis:** All of the participants will receive photobiomodulation in order to treat their periocular wrinkles. They will be using red and amber LEDs, with one color being used on each hemiface. The facial side to be treated with each color will be randomized. After an interval of 180 days, the participants will receive a cross-treatment. The primary variable of the study is the volume of periocular wrinkles (crow's feet) that will be measured by VisioFace® equipment. The secondary variables are elasticity (measured by Cutometer®) and hydration (measured by Corneometer®). Quality of life and self-assessment of the participants will be measured by using the adapted MelasQol-BP and Skindex-29 questionnaires. All of the variables will be measured before and after the group of 10 sessions. **Ethics and Dissemination:** This protocol was approved by the Research Ethics Committee of the Nove de Julho University (# 2.550.732). This trial has been registered in the Registro Brasileiro de Ensaios Clínicos (Brazilian Clinical Trials Registry) (REBEC Number: RBR-6YFCBM). This study is not recruiting yet.

Keywords: Skin aging, wrinkles, phototherapy, photobiomodulation, photodermatology.

WORD COUNT: 3891

STRENGTHS AND LIMITATIONS OF THIS STUDY:

- Each woman participating in this study will be evaluated before and after the treatment and the reduction of wrinkles will be measured;
- There is no control or placebo group, all of the participants will be treated. In this sense, each participant is in both the treatment group and the control group;
- This split-face study will eliminate the individual factors of each participant on the treatment outcomes;
- The VisioFace® equipment will standardize the parameters of acquiring the photographs, such as light exposure, and it will minimize bias;
- The habits of the participants may affect the results, as a consequence of their diet, their cosmetics use and their exposure to the sun.

INTRODUCTION

The skin covers the body and it has essential functions in order to maintain the homeostasis of the organism, presenting roles of defense, thermoregulation and sensory awareness. The maintenance of healthy skin and integrity are both extremely important.(1) Exposure to the sun speeds up the intrinsic ageing of the skin, due to the formation of free radicals and reactive oxygen species, as a result of UV radiation (1). Once UVA radiation penetrates deeper into the dermis, the resulting oxidative stress causes damage to the elastin fibers and collagen. In addition, there may occur a decrement of physiological antioxidant reserves and/or of a protective capacity of the skin.(2) The changes that are caused by aging modify the physical properties of the skin, leaving visible signs, such as epidermal hyperplasia, irregular pigmentation, telangiectasia, sagging tissues, a reduction of collagen and the elastin fibers, as well as a decrement of the natural moisturizing factor (NMF). These changes cause the appearance of expression lines and creases.(3,4)

Recent data from the Brazilian Institute of Geography and Statistics (IBGE) has shown that the average life expectancy of the Brazilian population has increased from 66 years in 1991 to 75 years in 2016. This is similar to the increase that has been verified on the worldwide population. The challenge faced by science in the last few years has been the development of procedures and technologies, with the aim of delaying the signs of aging and increasing the quality of life of elderly people, by achieving healthy skin.(5) Nowadays, the procedures in use aim to promote not only a cosmetic benefit, but also an improvement in the quality of the skin, increasing self-esteem, with a reduction of skin infections. As a result, these procedures can contribute to a longer and healthier life. Among the technologies being used to promote skin repair are dermo-cosmetics, as well as equipment, such as radiofrequency, phototherapy (intense pulsed light, LASER and light emitting diodes - LEDs) and microneedles.(6–9)

Phototherapy is a non-invasive procedure that has been used for tissue repair and healing.(10,11) The treatment is based upon the use of a light emitting device and the resulting pho-

tons are absorbed by the biological tissues, promoting photochemical, photophysical and photobiological actions. Phototherapy is not ablative, nor does it promote thermal effects, since the devices that are used in phototherapy are low powered LASERs and LEDs; i.e., there is no cutaneous damage and no need for any recovery time.(12) The LED devices are produced in a wide range of wavelengths, from UV through the visible to infrared spectrum (247 to 1300 nm). When compared to LASER, the LED devices have a lower cost and the practicality of being used in instruments that can illuminate larger surfaces. Studies have shown that LEDs can be used in therapeutical procedures with excellent results.(10,13,14) The use of LEDs in clinical practice has increased significantly and their main use has been in wound healing, tissue repair and rejuvenation, since they do not cause trauma or tissue destruction.(15) Some findings have suggested that if suitable parameters are used, the light acts on skin regeneration, by modulating cellular activity and collagen expression, with a decrease of the matrix metalloproteinases (MMP).(16) Usually, the wavelengths are chosen by the function that is needed for the purpose of the therapy. The wavelengths in the blue range (400 to 470 nm) are mainly used in the treatment of acne.(17) The wavelengths in the green range (500-570nm) have shown their ability to induce a proliferation of fibroblasts, as well as in the production and maturation of the collagen fibers.(18,19) The infrared range (700 to 1200nm) accelerates the healing process of lesions in the skin, increases the proliferation of cell differentiation, as well as contributing to an increase in the extracellular matrix.(20,21)

Many *in vitro, in vivo*, and clinical studies have demonstrated the anti-inflammatory, repair, skin rejuvenation and healing effects that are promoted by red light.(11,22) For an amber light, a study that was published by Smith in 2005 showed that it has an affinity for keratinocytes, melanocytes, as well as for the cells of Merkel and Langerhans, which are all of extreme importance in the maintenance of the epidermis.(23) Both of these wavelengths are absorbed by cytochrome c oxidase, however, it is considered that the red light penetrates deeper into the skin than does the amber light, due to the presence of melanin.(24–26) Given this background, this work will aim to evaluate the

percentages of reduction of the volume of periocular wrinkles when treated with red and amber LEDs.

METHODS AND ANALYSIS:

Study Design

This will be a controlled, randomized, double-blind, split-face, crossover, and unicentric clinical trial. This protocol has been written based upon the SPIRIT guidelines. The study will be performed in the ambulatory of Biophotonics at the Nove de Julho University (UNINOVE), São Paulo, Brazil. Dissemination and registration for participation in the study will be conducted through the website of the University of Nove de Julho (UNINOVE) and the recruited participants will mainly be residents of the city of São Paulo. The participants will be informed about the research, the procedures, the risks and the benefits and they will sign the informed consent form**.** The study was approved by the Research Ethics Committee of the Nove de Julho University on June 21th, 2017 (# 2.550.732). Only those participants who have read and have agreed to sign the informed consent form will be included in the study. The study will last for 2 years, with a start date of April 2018. The study is not recruiting yet.

The participants will answer an anamnesis questionnaire and a skin evalution will be performed by a medical doctor (ISD) searching for patients who meet the inclusion and exclusion criteria. Besides, They will receive regarding the importance of the use of sunscreen on skin health, preventing skin cancer and wrinkles.

Patient and Public Involvement Statement: Patients and or public were not involved in the design, recruitment to and conduct of the study.

Inclusion Criteria

This study will be conducted on women (40 to 65 years old), with skin phototypes II, III and IV on the Fitzpatrick scale and with signs of aging III and IV, on the Glogau scale.

Exclusion Criteria

This study will exclude any participants:

- With thyroid disorders (hyperthyroidism or hypothyroidism) and who are not undertaking the due treatment; or those that have been taking the medication for less than 1 year;
- Who have received a facial filling in the last 12 months;
- Who are doing any facial aesthetic procedure;
- Who are using retinoic acid or any vitamin A derivative (tretinoin, or isotretinoin, topical, or oral);
- Who are using cosmetics or medications that may increase the photosensitivity of the skin;
- Who present any pathology of the skin, such as acne, psoriasis, vitiligo, and so forth;
-Who presents big laterality of skin aging;
- Professional drivers;
- Who have undergone bariatric surgery or who are confined to a strict diet;
- Who are using any supplement (topic or oral) for the improvement of their skin condition;
- Who are pregnant or lactating;
- Who are not regular attendees for the treatments.

Sample Size Calculation

A pilot study with 10 patients was performed in order to generate the data for the sample size calculation. All of the participants of this pilot study signed the informed consent form. The largest and the smallest values of the percentages of reduction in the volume of the wrinkles for each treatment were obtained, as well as for the standard deviation of the measurements. The worst case sce-

narios were used for this calculation. The smallest and the largest values were 95 and 5, respectively; the highest standard deviation was 29 and the number of treatment groups was 2. These values were used for the calculation of the effect size, as follows:

$$\Delta = \frac{largest - smallest}{\left(\frac{\sigma}{\sqrt{n}}\right)^2} = \frac{95 - 5}{\left(\frac{29}{\sqrt{2}}\right)^2} = 0.214$$

By using the effect size value as calculated above, t-tests were used to evaluate the differences between the two dependent means (matched pairs); the test power was 80% and the one-tailed test was at a figure of 5%; the sample size calculated by G*Power Software (Version 3.1.9.2, Dusseldorf, Germany) was 137.

Randomization

The equalized randomization will be performed by a researcher (ACRTH) who is not directly involved in the treatment of the participants. It will be generated in Excel 2013 software (Microsoft, USA). The opaque envelopes will be marked and identified by sequential numbers and each envelope will receive a paper containing the information of which particular treatment will be performed in the right hemiface of the participant, in accordance with the draw. These envelopes will be sealed and securely stored in a safe place, under the utmost confidentiality, by the same researcher who generated the randomization. Immediately before the treatments, the researcher responsible for the treatment will receive the envelope, in sequence, and will then perform the indicated procedure.

Interventions

All of the participants will have their faces cleaned with a neutral cleansing soap and receive eye protection, followed by the LED application. The participants will have their eyes protected by

goggles, in order to safely allow for the illumination of their periocular region. This will also make the study blind, so that they do not know which wavelength is being applied to each hemiface. The application of phototherapy and the measurement of the parameters will be performed by ICSSR. Thus, this protocol will be a double-blind study.

All of the participants will receive photobiomodulation, in order to treat their periocular wrinkles, by using red and amber LEDs, with one color only at each hemiface. The facial side to be treated with each different color will be randomized. Group A will receive a red LED on the right side of their face and an amber LED on the left side of their face; Group B will receive a red LED on the left side of their face and an amber LED on the right side of their face. For both of the groups, the session will last for 10 minutes (5.4 J / cm² at each wavelength) and the complete treatment will be composed of 10 sessions, 2-3 sessions per week, within 1 month. After a period of 180 days, the crossover treatment will be performed: the participants in Group A will receive the application of an amber LED on their right hemiface and a red LED on their left hemiface, while the Group B participants will receive a treatment with a red LED on their right hemiface and an amber LED on their left hemiface. As performed in the first part of the study, both of the groups will have 10 minutes of exposure per session (5.4 J / cm² at each wavelength), with a complete treatment of 10 sessions, performed 2-3 sessions per week, within 1 month. All of the variables will be measured before and after the group of 10 sessions. This procedure will be conducted for each hemiface. This second group of data can inform if the effects of the first treatment would disappear after the washout period and if the clinical response to the second treatment would be different from the first one. The participants may not receive any other facial aesthetic procedure or any supplement (topic or oral) for the improvement of their skin condition during the development of this study.

Variables of the Study

The primary outcome of the study is the volume of wrinkles in the periocular region. The secondary outcomes are elasticity / sagging, hydration, melanin/spots, the quality of life, and the self-assessment by the participants.

The volume of wrinkles in the periocular region and the melanin spots: For the measurements of the primary variable, VisioFace® - RD (CK Electronic, Cologne, Germany) equipment will be used. This apparatus has a digital camera with white diode illumination that will record a standardized photograph of each participant's face. Through a computer program, parameters will be determined that will indicate the volume of wrinkles in the periocular region, which is commonly known as crow's feet.

Elasticity / Sagging: Other noninvasive measurements of the facial region will also be performed. The viscoelasticity of the skin will be evaluated in the periocular region by Cutometer® dual MPA 580 (CK Electronic) instrumentation. Cutaneous elasticity is an important parameter, because it provides indirect information regarding the quality and quantity of collagen and elastic fibers (structural fibers) that are degraded by the metalloproteinases. It is known that photoaged skin presents disorganized elastin and decreased collagen fibers.(27)

Hydration: Skin hydration will be evaluated by a Corneometer® - CM 825 (CK Electronic) probe. This parameter is related to the amount of water in the dermis and epidermis, which allows for suitable skin functions.(28)

Quality of life and self-assessment by the participants: The participants will respond to the Quality of Life and Self-Assessment Questionnaires. An interview of around 20 minutes will be enough to get all of the participant's answers. In terms of the quality of life, two adapted questionnaires will be

applied. First, an adaptation of the questionnaire for the quality of life of participants with dermatological diseases will be used (Skindex-29).(29) The second is the adapted version of the MELASQoL-BP questionnaire.(30) Due to the adaptations, these questionnaires will be evaluated in terms of reproducibility and internal consistency. For this, 20 participants, external to the main research, will respond to the questionnaires twice, with an interval of 30 days between the answers. Statistical analyzes will then be conducted. These particular participants will be duly informed about the research and, if they agree to participate in the study, they will sign the terms of free and informed consent. The questionnaire results for any rhytidectomy will be performed before and after the phototherapy treatments for the verification of the self-assessment by participants.(31)

All of the measurements will be performed by LRM, who was previously trained by the CK Electronic's representative in Brazil. The VisioFace® equipment has standardized illumination and face positioning in order to minimize any experimental bias. The questionnaires will be applied by LRM. The data that will be collected at this study will be managed only by the principal investigators (authors of this paper). The data will be saved on the University computer, protected by password.

The complete study timeline is presented at Figure 1.

Statistical Analyzes and Data Analyzes Plan

The Shapiro-Wilk test will be used to test for the normality of the data. If the data is non-parametric, the normalization will be performed by math strategy. The Student's t-test for dependent variables will be used for the inferential analyzes. A p-value < 0.05 will be considered statistically significant. DFTS will perform all of the statistical analyzes.

<Figure 1>

DISCUSSION

Photobiomodulation has been extensively studied for wound healing in the medical literature, showing good results.(32–34) The effect is related to the increased proliferation of dermal fibroblasts, collagen synthesis, decrease of the inflammatory cells and formation of the granulation tissue. Both, the use of LED and lasers have been considered effective. (35,36) Recently, some studies proposed the use of phototherapy in aesthetics protocols for rejuvenation. (15,37) However, it is still needed to optimize the parameters such as energy and number of sessions. (38)

Despite the fact that phototherapy has been proposed as an interesting tool to reduce wrinkles, clinical trials evaluating any real effects are sparse.(11,39,40) Regarding this subject, this study's protocol was designed in order to evaluate the reduction of wrinkles when using red or amber LED devices. This current work has described a study protocol for a unicentric randomized clinical trial based upon the comparison of two interventions. The study has been designed in order to optimize the obtainment of results and to minimize bias. Firstly, the participants will be their own control, since the measurements are going to be accomplished before and after a series of 10 sessions of interventions. Secondly, by performing a split-face study, this will eliminate the individual factors of each participant on the treatment outcomes. By being aware of preexisting systemic pathologies, personal daily care and food intake, together with smoking and drinking habits, may affect the results obtained. As a result, by performing both treatments on each patient, this will make these factors influence the results equally for the two groups. In addition, if the application of phototherapy generates some systemic effects, it will have the same influence on the results of the two treatments. This has been carefully considered, since some studies have shown systemic effects of phototherapy.(41,42).Thirdly, it is known that melanin absorbs light in the visible and infrared region of the spectra. Thus, the results of the application of both LED, in the red and amber, may be affected by the melanin content of the skin. In this sense, skin types II, III and IV were chosen as inclusion criteria in a way to standardize the melanin content of the participants, excluding the skin types with high melanin content (types V and VI) and the lower melanin content (Type I). Fourth, a double-

blind study will reduce errors of bias due to the subconscious influence of the volunteers, as well as the researchers on data acquisition. Finally, this randomization will be performed in an equalized way *(i.e., Group A = Group B)*, then, in the case of a patient's withdrawal after the first randomization, new inscriptions and a new randomization can be generated, thus allowing for the researchers to reach the desired number of patients for the study.

The aesthetics of the face may have positive or negative effects on the quality of life of the patients, as well as on their self-esteem. Despite being treated as futilities, aesthetic treatments may have strong and important influences on psychological and emotional levels, as well as on the well-being of the people. Some studies have already shown improvements in the quality of life and the self-assessment by patients after aesthetic treatments.(43,44) In this sense, the aforesaid questionnaires will be used in order to evaluate these aesthetic effects. Since the quality of life and the self-assessment questionnaires that have been validated in Portuguese when related to wrinkles are sparse, we decided to make fairly minor adaptions on some previously validated ones. The adaptation of the quality of life questionnaire for participants with dermatological diseases (29) has involved the removal of 10 questions related to skin diseases that are not suitable for participants with wrinkles, resulting in a new questionnaire with 19 questions. The adaptation of the quality of life questionnaire for wrinkles and for those participants with melasma - MelasQol-BP (30) has included the substitution of the word 'melasma' instead of the word wrinkles. Due to these adaptations, these questionnaires will be evaluated in terms of reproducibility and internal consistency, as has been performed on other questionnaires previously.(45)

When considering the importance of aesthetics of the face on the life role of a person, the development of efficacious treatments are essential. However, in order to prove that a treatment presents efficacy, the choice of a quantitative evaluation method is challenging. Most of the trials evaluating facial skin that can be found in the literature are based upon subjective measurements (patient satisfaction and photos), since quantitative studies use biopsies.(39,40,46) Here, for this research,

non-invasive quantification methods will be used aimed at evaluating a group of variables that may be affected or improved by photobiomodulation therapy (volume of wrinkles, elasticity/sagging and hydration). Treatment and evaluation procedures during this study will be performed by beauticians, since the procedures are noninvasive they are included in beauticians professional attributions. In case of the participants present any complication, ISD will evaluate the participant and guide the treatment.

Some studies have pointed to the efficacy of LEDS in tissue repair, cutaneous hydration and an increase in the production of the sustentation fibers. However, there are still studies that standardize dosimetry and the parameters of use.(47,48) Here, the choice of wavelengths was made from studies that had demonstrated that an application of red light on the skin could trigger cell proliferation, increased collagen fibers and decreased metalloproteinases; these studies had also demonstrated that an amber light can interact with the epidermal cells, triggering mitosis and cell renewal, as well as acting on the protection and the hydration of the epidermis; all of this information was together with clinical trials showing an improvement of wrinkles by using phototherapy.(11,15,22,40,49) There is no consensus in the literature regarding the washout period for phototherapy, which varies from 7 days to 12 weeks.(50–53) Due to this, a washout of 180 days for performing the crossover treatment was chosen, in order to evaluate if there were any residual effects from the first photobiomodulation treatment performed.

The results of this clinical trial may confirm the efficaciousness of phototherapy in reducing periocular wrinkles and show improvements of certain other parameters. Besides, the comparison between the reduction of wrinkles achieved by each wavelength may be a valuable contribution to the aesthetics area and on the way to developing new treatment protocols, with satisfactory results.

ETHICS AND DISSEMINATION:

This protocol was approved by the Research Ethics Committee of the Nove de Julho University

(Acceptance Number: 2.550.732). The trial has already been registered in the Registro Brasileiro de Ensaios Clínicos (REBEC Number: RBR-6YFCBM). and it grants public access to the full protocol. After publishing the protocol, the data will be collected and the results will be presented at conferences and published in a peer-reviewed Journal, selected by interest area and impact factor. At the end of the study, the main results will be disseminated to participants by email. The authorship of the results paper will include the authors of the protocol and others that may contribute to the procedures or analysis of data.

AUTHORS' CONTRIBUTIONS:

LRM wrote the protocol and will execute the measurements. LJM adapted the QoL and the self-assessment questionnaires and will perfom the reproducibility and internal consistency analysis and LRM will apply them to the participants. ISD designed the protocol and will evaluate the participants during the inclusion/exclusion criteria. DFTS performed the sample size calculation, proposed the statistics and will analyze the data. ACRTH generated the randomization and designed the study. CP conceived and designed the treatment protocol. All of the authors have read and approved the final version of the protocol and of the manuscript.

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COMPETING INTERESTS STATEMENT:

The authors declare the absence of any conflicts of interest.

FUNDING STATEMENT:

This research has received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

DATA SHARING STATEMENT:

Since this article is a study protocol there is no data colleted at the moment. However, the raw data that will be generated by this protocol will be shared at a repository for clinical data.

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Figure Captions:

Figure 1: Study Timeline.

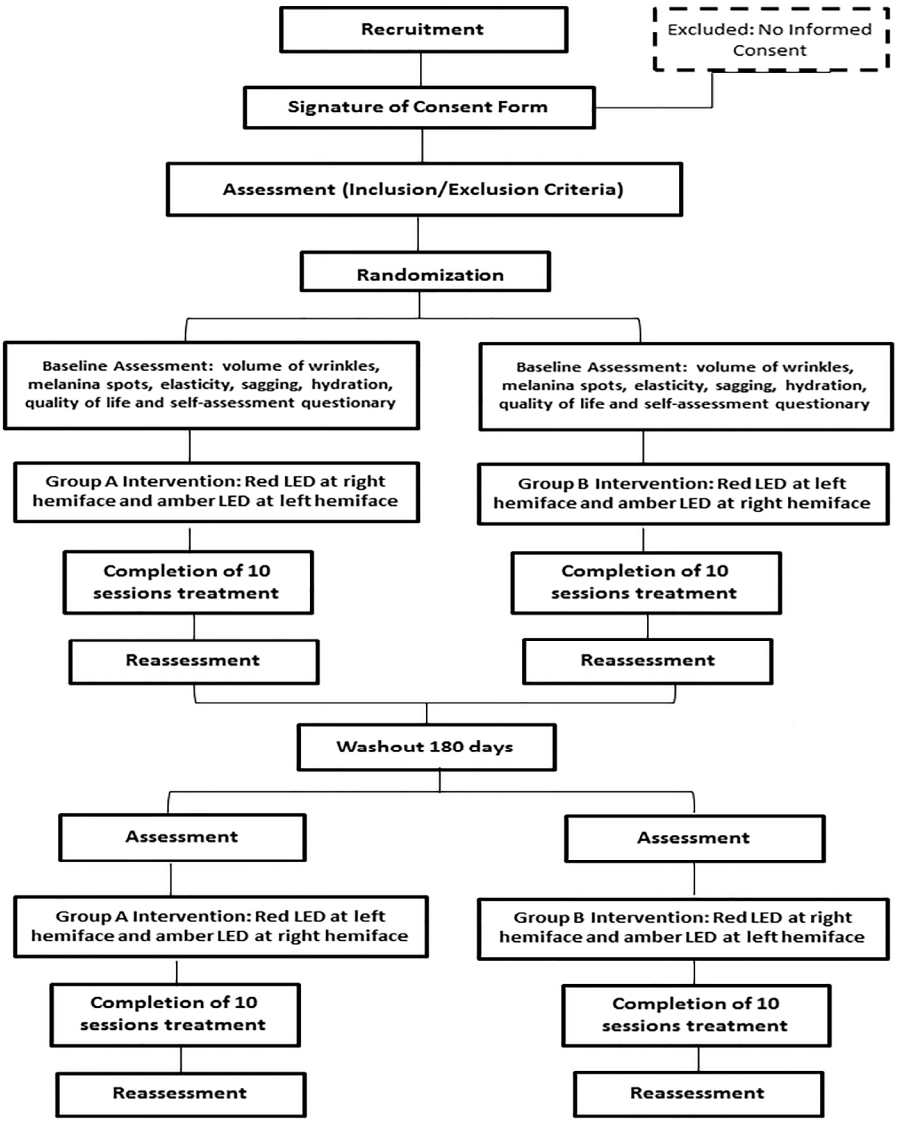

Figure 1. Study Timeline.

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	Page 1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	Page 2, 14
	2b	All items from the World Health Organization Trial Registration Data Set	N/A
Protocol version	3	Date and version identifier	Page 2
Funding	4	Sources and types of financial, material, and other support	N/A
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	Page 1 and 15
	5b	Name and contact information for the trial sponsor	N/A
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	N/A
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	Page 2, 4, 5
	6b	Explanation for choice of comparators	Page 5, 6
Objectives	7	Specific objectives or hypotheses	Page 2, 5
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	Page 6
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	Page 6
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	Page 6,7
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	Page 8, 9
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	N/A
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	Page 9, 10, 11
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	Page 9
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	Page 9, 10, 11
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Page 11

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	Page 7, 8
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	Page 6
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	Page 8
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	Page 8
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	Page 8, 9
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	Page 8, 9
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	Page 9, 10, 11
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	N/A

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	Page 11
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	Page 11
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	Page 10, 11
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	N/A
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	N/A
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	N/A
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	Page 8, 9, 10, 11
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	Page 2, 15
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	N/A

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	Page 6
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	Page 6
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	Page 16
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	Page 15
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	Page 15
	31b	Authorship eligibility guidelines and any intended use of professional writers	Page 15
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	Page 15
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Page 6
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Efficacy of phototherapy to treat facial aging when using a red versus amber LED: a protocol for a randomized controlled trial

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Keywords:	Sking aging, wrinkles, phototherapy, photobiomodulation, Photodermatology < DERMATOLOGY

Efficacy of phototherapy to treat facial aging when using a red versus amber LED: a protocol for a randomized controlled trial

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ABSTRACT

Introduction: The skin undergoes morphological and physiological changes with the advancing age of the individual. These changes may be caused by intrinsic and extrinsic factors that contribute to cellular aging and consequent skin aging. The term photoaging is used to characterize the aging of the skin that is caused by solar radiation. Clinically, the skin becomes more flaccid, thicker, and hyperpigmented, while there is an early appearance of wrinkles and other skin changes, such as skin cancer. Nowadays, there are numerous treatments for aging skin and one of them is with the use of phototherapy which uses light emitting diodes (LEDs). The objective of this study will be to evaluate the percentages of reduction of the volume of periocular wrinkles when treated with red and amber LEDs. **Methods and Analysis:** All of the participants will receive photobiomodulation in order to treat their periocular wrinkles. They will be using red and amber LEDs, with one color being used on each hemiface. The facial side to be treated with each color will be randomized. After an interval of 180 days, the participants will receive a cross-treatment. The primary variable of the study is the volume of periocular wrinkles (crow's feet) that will be measured by VisioFace® equipment. The secondary variables are elasticity (measured by Cutometer®) and hydration (measured by Corneometer®). Quality of life and self-assessment of the participants will be measured by using the adapted MelasQol-BP and Skindex-29 questionnaires. All of the variables will be measured before and after the group of 10 sessions. **Ethics and Dissemination:** This protocol was approved by the Research Ethics Committee of the Nove de Julho University (# 2.550.732). This trial has been registered in the Registro Brasileiro de Ensaios Clínicos (Brazilian Clinical Trials Registry) (REBEC Number: RBR-6YFCBM). This study is not recruiting yet.

Keywords: Skin aging, wrinkles, phototherapy, photobiomodulation, photodermatology.

WORD COUNT: 3891

STRENGTHS AND LIMITATIONS OF THIS STUDY:

- Each woman participating in this study will be evaluated before and after the treatment and the reduction of wrinkles will be measured;
- There is no control or placebo group, all of the participants will be treated. In this sense, each participant is in both the treatment group and the control group;
- This split-face study will eliminate the individual factors of each participant on the treatment outcomes;
- The VisioFace® equipment will standardize the parameters of acquiring the photographs, such as light exposure, and it will minimize bias;
- The habits of the participants may affect the results, as a consequence of their diet, their cosmetics use and their exposure to the sun.

INTRODUCTION

The skin covers the body and it has essential functions in order to maintain the homeostasis of the organism, presenting roles of defense, thermoregulation and sensory awareness. The maintenance of healthy skin and integrity are both extremely important.(1) Exposure to the sun speeds up the intrinsic ageing of the skin, due to the formation of free radicals and reactive oxygen species, as a result of UV radiation (1). Once UVA radiation penetrates deeper into the dermis, the resulting oxidative stress causes damage to the elastin fibers and collagen. In addition, there may occur a decrement of physiological antioxidant reserves and/or of a protective capacity of the skin.(2) The changes that are caused by aging modify the physical properties of the skin, leaving visible signs, such as epidermal hyperplasia, irregular pigmentation, telangiectasia, sagging tissues, a reduction of collagen and the elastin fibers, as well as a decrement of the natural moisturizing factor (NMF). These changes cause the appearance of expression lines and creases.(3,4)

Recent data from the Brazilian Institute of Geography and Statistics (IBGE) has shown that the average life expectancy of the Brazilian population has increased from 66 years in 1991 to 75 years in 2016. This is similar to the increase that has been verified on the worldwide population. The challenge faced by science in the last few years has been the development of procedures and technologies, with the aim of delaying the signs of aging and increasing the quality of life of elderly people, by achieving healthy skin.(5) Nowadays, the procedures in use aim to promote not only a cosmetic benefit, but also an improvement in the quality of the skin, increasing self-esteem, with a reduction of skin infections. As a result, these procedures can contribute to a longer and healthier life. Among the technologies being used to promote skin repair are dermo-cosmetics, as well as equipment, such as radiofrequency, phototherapy (intense pulsed light, LASER and light emitting diodes - LEDs) and microneedles.(6–9)

Phototherapy is a non-invasive procedure that has been used for tissue repair and healing.(10,11) The treatment is based upon the use of a light emitting device and the resulting pho-

tons are absorbed by the biological tissues, promoting photochemical, photophysical and photobiological actions. Phototherapy is not ablative, nor does it promote thermal effects, since the devices that are used in phototherapy are low powered LASERs and LEDs; i.e., there is no cutaneous damage and no need for any recovery time.(12) The LED devices are produced in a wide range of wavelengths, from UV through the visible to infrared spectrum (247 to 1300 nm). When compared to LASER, the LED devices have a lower cost and the practicality of being used in instruments that can illuminate larger surfaces. Studies have shown that LEDs can be used in therapeutical procedures with excellent results.(10,13,14) The use of LEDs in clinical practice has increased significantly and their main use has been in wound healing, tissue repair and rejuvenation, since they do not cause trauma or tissue destruction.(15) Some findings have suggested that if suitable parameters are used, the light acts on skin regeneration, by modulating cellular activity and collagen expression, with a decrease of the matrix metalloproteinases (MMP).(16) Usually, the wavelengths are chosen by the function that is needed for the purpose of the therapy. The wavelengths in the blue range (400 to 470 nm) are mainly used in the treatment of acne.(17) The wavelengths in the green range (500-570nm) have shown their ability to induce a proliferation of fibroblasts, as well as in the production and maturation of the collagen fibers.(18,19) The infrared range (700 to 1200nm) accelerates the healing process of lesions in the skin, increases the proliferation of cell differentiation, as well as contributing to an increase in the extracellular matrix.(20,21)

Many *in vitro, in vivo*, and clinical studies have demonstrated the anti-inflammatory, repair, skin rejuvenation and healing effects that are promoted by red light.(11,22) For an amber light, a study that was published by Smith in 2005 showed that it has an affinity for keratinocytes, melanocytes, as well as for the cells of Merkel and Langerhans, which are all of extreme importance in the maintenance of the epidermis.(23) Both of these wavelengths are absorbed by cytochrome c oxidase, however, it is considered that the red light penetrates deeper into the skin than does the amber light, due to the presence of melanin.(24–26) Given this background, this work will aim to evaluate the

percentages of reduction of the volume of periocular wrinkles when treated with red and amber LEDs.

METHODS AND ANALYSIS:

Study Design

This will be a controlled, randomized, double-blind, split-face, crossover, and unicentric clinical trial. This protocol has been written based upon the SPIRIT guidelines. The study will be performed in the ambulatory of Biophotonics at the Nove de Julho University (UNINOVE), São Paulo, Brazil. Dissemination and registration for participation in the study will be conducted through the website of the University of Nove de Julho (UNINOVE) and the recruited participants will mainly be residents of the city of São Paulo. The participants will be informed about the research, the procedures, the risks and the benefits and they will sign the informed consent form**.** The study was approved by the Research Ethics Committee of the Nove de Julho University on June 21th, 2017 (# 2.550.732). Only those participants who have read and have agreed to sign the informed consent form will be included in the study. The study will last for 2 years, with a start date of May 2018. The study is not recruiting yet.

After recruitment, the researcher will check if the patient meets inclusion/exclusion criteria based on anamnesis and skin evaluation. Anamnesis is an interview performed by the health professional in order to know the patient medical and aesthetics treatment history, as well as daily personal and social habits, which may have any influence in treatment outcome. Regarding the daily personal and social habits, anamnesis will include information regarding sun exposure, smoking and drinking frequency, sleep quality, dietary habits, water intake, professional aesthetics treatment on the face and homecare cosmetics use. Anamnesis was not validated since it is not an instrument to measure patient outcome.(27) Skin evaluation, which will be performed by a medical doctor (ISD), includes skin phototype and degree of wrinkles severity. Patients will receive information regarding the importance of the use of sunscreen on skin health, preventing skin cancer and wrinkles.

Patient and Public Involvement Statement: Patients and or public were not involved in the design, recruitment to and conduct of the study.

Inclusion Criteria

This study will be conducted on women (40 to 65 years old), with skin phototypes II, III and IV on the Fitzpatrick scale and with signs of aging III and IV, on the Glogau scale.

Exclusion Criteria

This study will exclude any participants:

- With thyroid disorders (hyperthyroidism or hypothyroidism) and who are not undertaking the due treatment; or those that have been taking the medication for less than 1 year;
- Who have received a facial filling in the last 12 months;
- Who are doing any facial aesthetic procedure;
- Who are using retinoic acid or any vitamin A derivative (tretinoin, or isotretinoin, topical, or oral);
- Who are using cosmetics or medications that may increase the photosensitivity of the skin;
- Who present any pathology of the skin, such as acne, psoriasis, vitiligo, and so forth;
-Who presents big laterality of skin aging;
- Professional drivers;
- Who have undergone bariatric surgery or who are confined to a strict diet;
- Who are using any supplement (topic or oral) for the improvement of their skin condition;
- Who are pregnant or lactating;
- Who are not regular attendees for the treatments.

Sample Size Calculation

A pilot study with 10 patients was performed in order to generate the data for the sample size calculation. All of the participants of this pilot study signed the informed consent form. The largest and the smallest values of the percentages of reduction in the volume of the wrinkles for each treatment were obtained, as well as for the standard deviation of the measurements. The worst case scenarios were used for this calculation. The smallest and the largest values were 95 and 5, respectively; the highest standard deviation was 29 and the number of treatment groups was 2. These values were used for the calculation of the effect size, as follows:

$$\Delta = \frac{largest - smallest}{\left(\frac{\sigma}{\sqrt{n}}\right)^2} = \frac{95 - 5}{\left(\frac{29}{\sqrt{2}}\right)^2} = 0.214$$

By using the effect size value as calculated above, t-tests were used to evaluate the differences between the two dependent means (matched pairs); the test power was 80% and the one-tailed test was at a figure of 5%; the sample size calculated by G*Power Software (Version 3.1.9.2, Dusseldorf, Germany) was 137.

Randomization

The equalized randomization will be performed by a researcher (ACRTH) who is not directly involved in the treatment of the participants. It will be generated in Excel 2013 software (Microsoft, USA). The opaque envelopes will be marked and identified by sequential numbers and each envelope will receive a paper containing the information of which particular treatment will be performed in the right hemiface of the participant, in accordance with the draw. These envelopes will be sealed and securely stored in a safe place, under the utmost confidentiality, by the same researcher who

generated the randomization. Immediately before the treatments, the researcher responsible for the treatment will receive the envelope, in sequence, and will then perform the indicated procedure.

Interventions

All of the participants will have their faces cleaned with a neutral cleansing soap and receive eye protection, followed by the LED application. The participants will have their eyes protected by goggles, in order to safely allow for the illumination of their periocular region. This will also make the study blind, so that they do not know which wavelength is being applied to each hemiface. The application of phototherapy and the measurement of the parameters will be performed by ICSSR. Thus, this protocol will be a double-blind study.

All of the participants will receive photobiomodulation, in order to treat their periocular wrinkles, by using red and amber LEDs, with one color only at each hemiface. The facial side to be treated with each different color will be randomized. Group A will receive a red LED on the right side of their face and an amber LED on the left side of their face; Group B will receive a red LED on the left side of their face and an amber LED on the right side of their face. For both of the groups, the session will last for 10 minutes (5.4 J / cm² at each wavelength) and the complete treatment will be composed of 10 sessions, 2-3 sessions per week, within 1 month. After a period of 180 days, the crossover treatment will be performed: the participants in Group A will receive the application of an amber LED on their right hemiface and a red LED on their left hemiface, while the Group B participants will receive a treatment with a red LED on their right hemiface and an amber LED on their left hemiface. As performed in the first part of the study, both of the groups will have 10 minutes of exposure per session (5.4 J / cm² at each wavelength), with a complete treatment of 10 sessions, performed 2-3 sessions per week, within 1 month. All of the variables will be measured before and after the group of 10 sessions. This procedure will be conducted for each hemiface. This second group of data can inform if the effects of the first treatment would disappear after the washout period and if

the clinical response to the second treatment would be different from the first one. The participants may not receive any other facial aesthetic procedure or any supplement (topic or oral) for the improvement of their skin condition during the development of this study.

Variables of the Study

The primary outcome of the study is the volume of wrinkles in the periocular region. The secondary outcomes are elasticity / sagging, hydration, melanin/spots, the quality of life, and the self-assessment by the participants.

The volume of wrinkles in the periocular region and the melanin spots: For the measurements of the primary variable, VisioFace® - RD (CK Electronic, Cologne, Germany) equipment will be used. This apparatus has a digital camera with white diode illumination that will record a standardized photograph of each participant's face. Through a computer program, parameters will be determined that will indicate the volume of wrinkles in the periocular region, which is commonly known as crow's feet.

Elasticity / Sagging: Other noninvasive measurements of the facial region will also be performed. The viscoelasticity of the skin will be evaluated in the periocular region by Cutometer® dual MPA 580 (CK Electronic) instrumentation. Cutaneous elasticity is an important parameter, because it provides indirect information regarding the quality and quantity of collagen and elastic fibers (structural fibers) that are degraded by the metalloproteinases. It is known that photoaged skin presents disorganized elastin and decreased collagen fibers.(28)

Hydration: Skin hydration will be evaluated by a Corneometer® - CM 825 (CK Electronic) probe. This parameter is related to the amount of water in the dermis and epidermis, which allows for suitable skin functions.(29)

Quality of life and self-assessment by the participants: The participants will respond to the Quality of Life and Self-Assessment Questionnaires. An interview of around 20 minutes will be enough to get all of the participant's answers. In terms of the quality of life, two adapted questionnaires will be applied. First, an adaptation of the questionnaire for the quality of life of participants with dermatological diseases will be used (Skindex-29).(30) The second is the adapted version of the ME-LASQoL-BP questionnaire.(31) Due to the adaptations, these questionnaires will be evaluated in terms of reproducibility and internal consistency. For this, 20 participants, external to the main research, will respond to the questionnaires twice, with an interval of 30 days between the answers. Statistical analyzes will then be conducted. These particular participants will be duly informed about the research and, if they agree to participate in the study, they will sign the terms of free and informed consent. The questionnaire results for any rhytidectomy will be performed before and after the phototherapy treatments for the verification of the self-assessment by participants.(32)

All of the measurements will be performed by LRM, who was previously trained by the CK Electronic's representative in Brazil. The VisioFace® equipment has standardized illumination and face positioning in order to minimize any experimental bias. The questionnaires will be applied by LRM. The data that will be collected at this study will be managed only by the principal investigators (authors of this paper). The data will be saved on the University computer, protected by password.

The complete study timeline is presented at Figure 1.

Statistical Analyzes and Data Analyzes Plan

The Shapiro-Wilk test will be used to test for the normality of the data. If the data is non-parametric, the normalization will be performed by math strategy. The Student's t-test for dependent variables will be used for the inferential analyzes. A p-value < 0.05 will be considered statistically significant. DFTS will perform all of the statistical analyzes.

<Figure 1>

DISCUSSION

Photobiomodulation has been extensively studied for wound healing in the medical literature, showing good results.(33–35) The effect is related to the increased proliferation of dermal fibroblasts, collagen synthesis, decrease of the inflammatory cells and formation of the granulation tissue. Both, the use of LED and lasers have been considered effective. (36,37) Recently, some studies proposed the use of phototherapy in aesthetics protocols for rejuvenation. (15,38) However, it is still needed to optimize the parameters such as energy and number of sessions. (39)

Despite the fact that phototherapy has been proposed as an interesting tool to reduce wrinkles, clinical trials evaluating any real effects are sparse.(11,40,41) Regarding this subject, this study's protocol was designed in order to evaluate the reduction of wrinkles when using red or amber LED devices. This current work has described a study protocol for a unicentric randomized clinical trial based upon the comparison of two interventions. The study has been designed in order to optimize the obtainment of results and to minimize bias. Firstly, the participants will be their own control, since the measurements are going to be accomplished before and after a series of 10 sessions of interventions. Secondly, by performing a split-face study, this will eliminate the individual factors of each participant on the treatment outcomes. By being aware of preexisting systemic pathologies, personal daily care and food intake, together with smoking and drinking habits, may affect the results obtained. As a result, by performing both treatments on each patient, this will make these factors

influence the results equally for the two groups. In addition, if the application of phototherapy generates some systemic effects, it will have the same influence on the results of the two treatments. This has been carefully considered, since some studies have shown systemic effects of phototherapy.(42,43).Thirdly, it is known that melanin absorbs light in the visible and infrared region of the spectra. Thus, the results of the application of both LED, in the red and amber, may be affected by the melanin content of the skin. In this sense, skin types II, III and IV were chosen as inclusion criteria in a way to standardize the melanin content of the participants, excluding the skin types with high melanin content (types V and VI) and the lower melanin content (Type I). Fourth, a double-blind study will reduce errors of bias due to the subconscious influence of the volunteers, as well as the researchers on data acquisition. Finally, this randomization will be performed in an equalized way *(i.e., Group A = Group B)*, then, in the case of a patient's withdrawal after the first randomization, new inscriptions and a new randomization can be generated, thus allowing for the researchers to reach the desired number of patients for the study.

The aesthetics of the face may have positive or negative effects on the quality of life of the patients, as well as on their self-esteem. Despite being treated as futilities, aesthetic treatments may have strong and important influences on psychological and emotional levels, as well as on the well-being of the people. Some studies have already shown improvements in the quality of life and the self-assessment by patients after aesthetic treatments.(44,45) In this sense, the aforesaid questionnaires will be used in order to evaluate these aesthetic effects. Since the quality of life and the self-assessment questionnaires that have been validated in Portuguese when related to wrinkles are sparse, we decided to make fairly minor adaptions on some previously validated ones. The adaptation of the quality of life questionnaire for participants with dermatological diseases (30) has involved the removal of 10 questions related to skin diseases that are not suitable for participants with wrinkles, resulting in a new questionnaire with 19 questions. The adaptation of the quality of life questionnaire for wrinkles and for those participants with melasma - MelasQol-BP (31) has included

the substitution of the word 'melasma' instead of the word wrinkles. Due to these adaptations, these questionnaires will be evaluated in terms of reproducibility and internal consistency, as has been performed on other questionnaires previously.(46)

When considering the importance of aesthetics of the face on the life role of a person, the development of efficacious treatments are essential. However, in order to prove that a treatment presents efficacy, the choice of a quantitative evaluation method is challenging. Most of the trials evaluating facial skin that can be found in the literature are based upon subjective measurements (patient satisfaction and photos), since quantitative studies use biopsies.(40,41,47) Here, for this research, non-invasive quantification methods will be used aimed at evaluating a group of variables that may be affected or improved by photobiomodulation therapy (volume of wrinkles, elasticity/sagging and hydration). Treatment and evaluation procedures during this study will be performed by beauticians, since the procedures are noninvasive they are included in beauticians professional attributions. In case of the participants present any complication, ISD will evaluate the participant and guide the treatment.

Some studies have pointed to the efficacy of LEDS in tissue repair, cutaneous hydration and an increase in the production of the sustentation fibers. However, there are still studies that standardize dosimetry and the parameters of use.(48,49) Here, the choice of wavelengths was made from studies that had demonstrated that an application of red light on the skin could trigger cell proliferation, increased collagen fibers and decreased metalloproteinases; these studies had also demonstrated that an amber light can interact with the epidermal cells, triggering mitosis and cell renewal, as well as acting on the protection and the hydration of the epidermis; all of this information was together with clinical trials showing an improvement of wrinkles by using phototherapy.(11,15,22,41,50) There is no consensus in the literature regarding the washout period for phototherapy, which varies from 7 days to 12 weeks.(51–54) Due to this, a washout of 180 days for performing the crossover

treatment was chosen, in order to evaluate if there were any residual effects from the first photobiomodulation treatment performed.

The results of this clinical trial may confirm the efficaciousness of phototherapy in reducing periocular wrinkles and show improvements of certain other parameters. Besides, the comparison between the reduction of wrinkles achieved by each wavelength may be a valuable contribution to the aesthetics area and on the way to developing new treatment protocols, with satisfactory results.

ETHICS AND DISSEMINATION:

This protocol was approved by the Research Ethics Committee of the Nove de Julho University

(Acceptance Number: 2.550.732). The trial has already been registered in the Registro Brasileiro de Ensaios Clínicos (REBEC Number: RBR-6YFCBM). and it grants public access to the full protocol. After publishing the protocol, the data will be collected and the results will be presented at conferences and published in a peer-reviewed Journal, selected by interest area and impact factor. At the end of the study, the main results will be disseminated to participants by email. The authorship of the results paper will include the authors of the protocol and others that may contribute to the procedures or analysis of data.

AUTHORS' CONTRIBUTIONS:

LRM wrote the protocol and will execute the measurements. LJM adapted the QoL and the self-assessment questionnaires and will perfom the reproducibility and internal consistency analysis and LRM will apply them to the participants. ISD designed the protocol and will evaluate the participants during the inclusion/exclusion criteria. DFTS performed the sample size calculation, proposed the statistics and will analyze the data. ACRTH generated the randomization and designed the study.

CP conceived and designed the treatment protocol. All of the authors have read and approved the final version of the protocol and of the manuscript.

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COMPETING INTERESTS STATEMENT:

The authors declare the absence of any conflicts of interest.

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DATA SHARING STATEMENT:

Since this article is a study protocol there is no data colleted at the moment. However, the raw data that will be generated by this protocol will be shared at a repository for clinical data.

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Figure Captions:

Figure 1: Study Timeline.

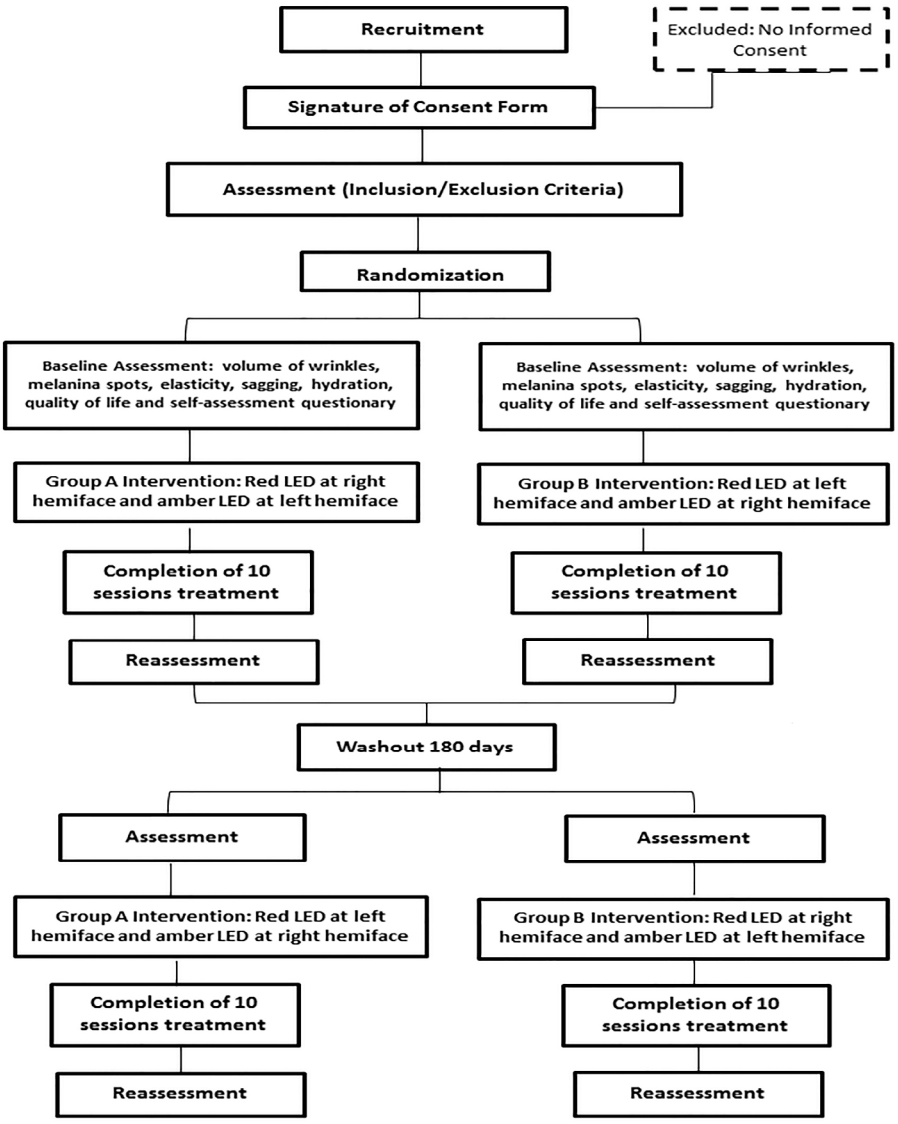

Figure 1. Study Timeline.

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	Page 1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	Page 2, 14
	2b	All items from the World Health Organization Trial Registration Data Set	N/A
Protocol version	3	Date and version identifier	Page 2
Funding	4	Sources and types of financial, material, and other support	N/A
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	Page 1 and 15
	5b	Name and contact information for the trial sponsor	N/A
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	N/A
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	Page 2, 4, 5
	6b	Explanation for choice of comparators	Page 5, 6
Objectives	7	Specific objectives or hypotheses	Page 2, 5
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	Page 6
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	Page 6
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	Page 6,7
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	Page 8, 9
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	N/A
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	Page 9, 10, 11
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	Page 9
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	Page 9, 10, 11
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Page 11

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	Page 7, 8
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	Page 6
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	Page 8
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	Page 8
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	Page 8, 9
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	Page 8, 9
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	Page 9, 10, 11
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	N/A

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	Page 11
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	Page 11
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	Page 10, 11
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	N/A
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	N/A
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	N/A
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	Page 8, 9, 10, 11
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	Page 2, 15
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	N/A

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	Page 6
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	Page 6
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	Page 16
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	Page 15
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	Page 15
	31b	Authorship eligibility guidelines and any intended use of professional writers	Page 15
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	Page 15
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Page 6
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.